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DETERMINATION OF SOTALOL, OXPRENOLOL AND LABETALOL IN BINARY MIXTURES AND IN SPIKED HUMAN SERUM BY DERIVATIVE SPECTROPHOTOMETRIC METHOD

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Abstract: The usefulness of derivative spectrophotometry for the determination of labetalol, sotalol and oxprenolol in binary mixtures and in human spiked serum was checked. To this aim a spectrophotometric analysis of samples in the UV range was carried out and the obtained results revealed that derivative spectrophotometry allows for the fast, accurate and precise determination of the tested substances in spite of their clear interference in the zero-order spectra. For quantitative determinations "zero-crossing" technique was used to establish wavelengths for zeros of specified component. In a mixture of labetalol and oxprenolol the following wavelengths were established: D1 λ = 245.32 nm and 266.03 nm, D2 λ = 243.30 nm and 301.09 nm. respectively. D3 derivative did not show zeros suitable for quantitative analysis. For the analysis of labetalol and sotalol mixture, D3 derivative spectrophotometry was used at the following wavelengths: λ = 246.03 nm and λ = 249.91 nm, respectively. In this case, the curves of D1 and D2 derivatives showed no zeros that can be used in quantitative analysis. To determine the concentration of the components in a mixture containing oxprenolol and sotalol the following wavelengths were selected: for oxprenolol D1 λ = 245.32 nm, D2 λ = 240.18 nm, D3 λ = 232.05 nm and for sotalol D1 λ = 230.56 nm, D2 λ = 232.65 nm and D3 λ = 238.84 nm, respectively. The developed spectrophotometric method was characterized by high sensitivity and accuracy, LOD determined for sotalol was in the range of 0.21-1.88 μg/mL, for labetalol 1.00-3.43 μg/mL and for oxprenolol 0.16-2.06 μg/mL; LOQ determined for sotalol was in the range of 0.65-5.70 μg/mL, for labetalol 3.11-10.39 μg/mL and for oxprenolol 0.47-6.23 μg/mL, depending on the composition of the tested mixture and the order of the derivative. The recovery of the individual components was within the range of 100 ± 5%. The linearity range was wide and estimated for sotalol in the range of 11.00-38.50 μg/mL, for labetalol 12.80-44.80 μg/mL and for oxprenolol 12.60-44.10 μg/mL with correlation coefficients in the range of 0.9977-0.9999.

Keywords: sotalol, oxprenolol, labetalol, derivative spectrophotometry, spiked human serum

Abbreviations: ICH – International Conference on Harmonisation, LAB – labetalol, OXP – oxprenolol, SOT – sotalol

Sotalol (SOT), (RS)-N-{4-[1-hydroxy-2-(propan-2-ylamino)ethyl]phenyl} methanesulfonamide (Fig. 1A), is a nonselective antagonist of β-adrenergic receptors. It has β-adrenolytic activity, prolongs the repolarization of the heart. Sotalol strongly acts in ventricular arrhythmias, less in the termination of atrial fibrillation and atrial flutter. Due to the fact that it lengthens the duration of action potential and repolarization of cardiac cells, it belongs to the group of antiarrhythmic drugs. It reduces the risk of recurrent myocardial infarction, without affecting mortality.

Oxprenolol (OXP), (RS)-1-[2-(allyloxy)phenoxy]-3-(isopropylamino)propan-2-ol (Fig. 1B), belongs to the group of non-selective β-blockers. It has medium sympathomimetic activity. Oxprenolol is safe when used in ischemic heart disease accompanied by heart failure due to its intrinsic activity, it slows the heart rate, reduces oxygen demand, and improves exercise tolerance. It is mainly used in patients with hypertension, ischemic heart disease, as well as in acute myocardial infarction.

Labetalol (LAB), (RS)-2-hydroxy-5-{1-hydroxy-2-[(1-methyl-3-phenylpropyl)amino] ethyl} benzamide (Fig. 1C), is a competitive antagonist of α1 and β receptors. Blocking the receptor α1 causes smooth muscle relaxation and dilation of the arteries, and that is why labetalol is mainly used to

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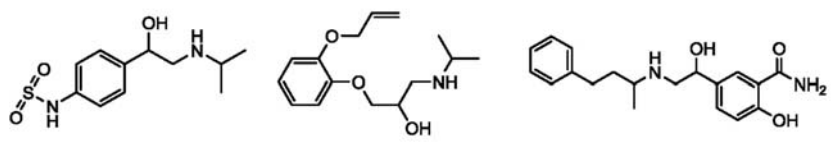
lower blood pressure. It is recommended in cases where hypertension occurs in conjunction with coronary heart disease and with increased plasma renin activity (1, 2).

Due to the extensive use of drugs from the group of β-blockers in the treatment of hypertension and other cardiovascular diseases, there are many studies on the analysis of this class of drugs available in the literature. They are mostly based on separation methods such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), liquid chromatography (LC) and gas chromatography (GC) (3-7). For the determination of sotalol and other β-blockers in serum and urine LC-MS method or capillary electrophoresis (CE) with amperometric detection were used (8). These drugs may appear in different enantiomeric forms that can vary in pharmacological activity and toxicity and for their analysis RP-LC (reversed-phase liquid chromatography) (9) and methods based on capillary electrophoresis were used (10-13).

The use of β-blockers in sport as substances enhancing physical fitness has led to the need for the application of analytical methods in antidoping control (14, 15).

In the comprehensive analysis of selected substances of this group, spectrophotometric method was used, in which chemometric methods were applied to eliminate the interferences in spectra of tested substances (16). Spectrophotometric methods are found to be preferable instead of hyphenated analytical instrumentations or techniques such as LC/MS and GC/MS due to the fast quantitative resolution of samples containing two or more substances without needing any special chemical pretreatment. In our previous studies, we have applied a derivative spectrophotometric method for the determination of other substances from group of hypotensive drugs in their mixtures, in the presence of substances having an influence on the proper function of cardiovascular system (17-19).

The widespread use of drugs from the group of β-blockers led the authors to develop a direct, rapid and accurate derivative spectrophotometric method for the determination of SOT, OXP and LAB both in mixtures and in biological material.



A B C

Figure 1. Chemical structure (A) sotalol (SOT), (B) oxprenolol (OXP) and (C) labetalol (LAB)

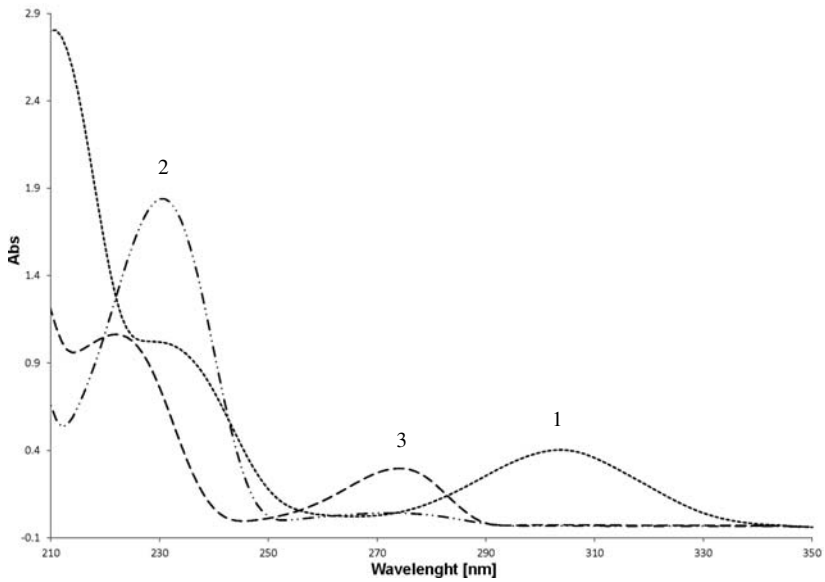


Figure 2. Zero-order absorption spectra of LAB (1- c = 38.4 mg/mL), SOT (2 - c = 38.4 mg/mL) and OXP (3 - c = 44.1 mg/mL)

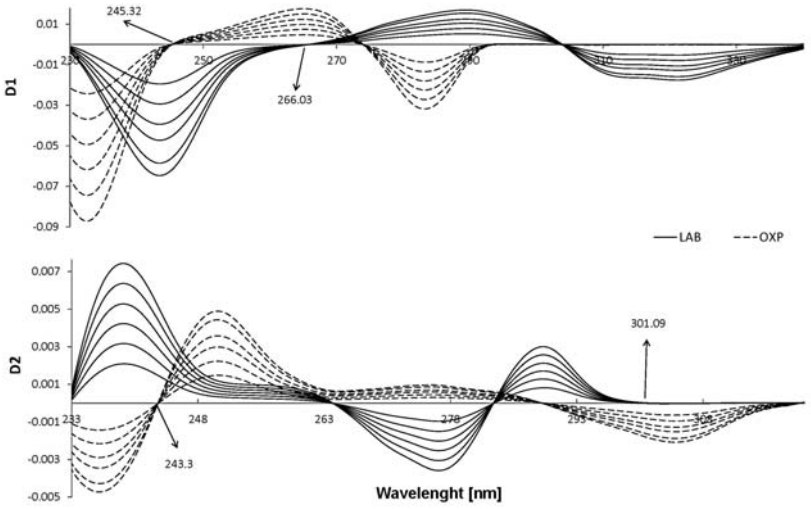


Figure 3. The first (D1) and second (D2) derivative values for standard solutions of LAB (-) and OXP(——)

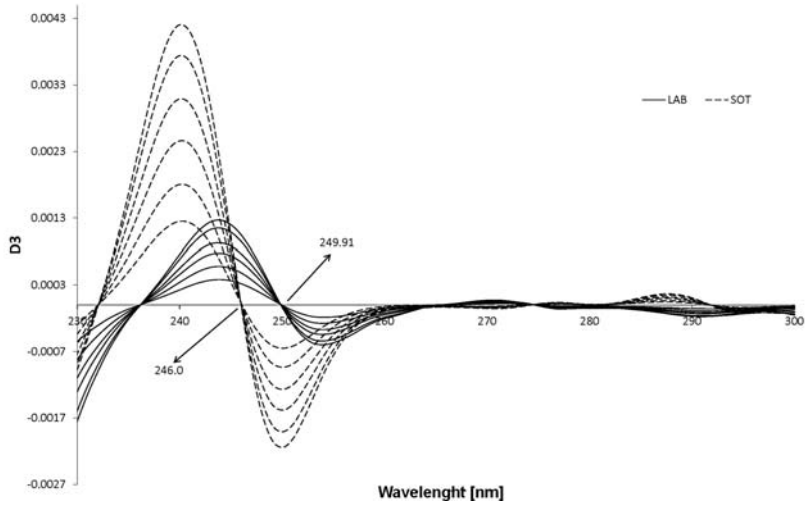


Figure 4. The third (D3) derivative values for standard solutions of LAB (-) and SOT(——).

EXPERIMENTAL

Instruments

Spectrophotometer UV-VIS Cary 100 (Varian), quartz cuvettes (l = 1 cm). Computer Dell Optiplex 755; Intel(R) Core(TM)2 Duo CPU; E4500 @ 2.20 GHz; 1.18 GHz, 1.95 GB Ram (Microsoft Office 2010, Statistica 10, edition 2012).

Chemicals

Reference standards:

Sotalol hydrochloride – European Pharmacopoeia (EP) Reference Standard oxprenolol hydrochloride – European Pharmacopoeia (EP) Reference Standard, labetalol hydrochloride – European Pharmacopoeia (EP) Reference Standard (all from Fluka). Reagents of analytical grade quality: methanol. Human serum was obtained by courtesy of the Regional Blood Transfusion Center in Kraków.

Procedures

Standard and working solution

SOT - to a 10.0 mL volumetric flask 5.5 mg of sotalol hydrochloride was weighed using analytical balance, and filled with methanol to a specified volume.

Table 1. Validation parameters of the proposed spectrophotometric methods for the determination of sotalol.

Parameters	Sotalol hydrochloride			
	D1 (λ = 230.56nm)	D2 (λ = 232.65 nm)	D3 (λ = 238.84 nm)	D3 (λ = 249.91 nm)
Linearity range [g/mL]	11.00 - 38.50			
Regression equation	$y = 0.0021x + 0.0010$ $y = 1.0 \cdot 10^{-6}x^2 + 2.1\ 10^{-3}x - 3.4 \cdot 10^{-4}$	$y = 0.0005x + 0.0002$	$y = 0.0001x + 0.2 \cdot 10^{-4}$	$y = 0.00006x + 0.4 \cdot 10^{-4}$
Correlation coefficient (r)	0.9999	0.9986	0.9989	0.9991
Durbin-Watson test	1.508*	1.968	1.919	1.919
Shapiro-Wilk test (p)	0.978 (0.941)*	0.990 (0.990)	0.972 (0.905)	0.986 (0.976)
Mandel's test (p)	14.918 (0.031)	1.295 (0.008)	0.916 (0.409)	0.310 (0.335)
LOD [g/mL]	0.21	1.88	1.85	1.51
LOQ [g/mL]	0.65	5.70	5.60	4.56
Accuracy[a] [%]	101.77 ± 1.88	101.00 ± 1.60	99.77 ± 1.17	100.75 ± 2.15
Precision[b] [%]	102.35	101.56	100.25	99.85
RSD%	1.84	1.58	1.17	2.14

[a)] Three concentrations of each analyte (17.60, 22.00, 26.40 µg/mL), repeated three times for each concentrate. [b)] Three concentrations of each analyte (11.00, 22.00, 33.00 µg/mL), repeated three times for each concentrate. * for quadratic equation.

OXP - to a 10.0 mL volumetric flask 6.3 mg of oxprenolol hydrochloride was weighed using analytical balance, and filled with methanol to a specified volume.

LAB - to a 10.0 mL volumetric flask 6.4 mg of labetalol hydrochloride was weighed using analytical balance, and filled with methanol to a specified volume.

For direct measurements, standard solutions were diluted with methanol in a 5.0 mL volumetric flask, to obtain working solutions at a concentrations in the range from 11.00 to 38.50 µg/mL for SOT, from 12.60 to 44.10 µg/mL for OXP and from 12.80 to 44.80 µg/mL for LAB.

Spectral characteristics of SOT, OXP and LAB

In the first stage of the study, absorption spectra of the standard solutions of SOT (38.50 µg/mL), OXP (44.10 µg/mL) and LAB (38.40 µg/mL) were recorded in quartz cuvettes (l = 1 cm), using methanol as a reference solution in the UV range. The registered spectra were characterized by a not very specific course and clear interference was observed in the range 215-290 nm. Analysis of the spectra led to the conclusion that the direct determination of the tested substances, based on zero-order absorption spectra is much more difficult, and thus results obtained in this way because of the interference may be burdened with a significant error (Fig. 2).

Conversion of zero-order spectra into derivatives caused their significant variation. Applying „zero-crossing" technique allowed to determine the wavelengths that were used for quantitative analysis of the individual components of the tested mixtures. For a mixture of LAB and OXP the first derivative of the spectrum (D1) and the second derivative of the spectrum (D2) were useful for quantitative analysis. For the determination of LAB the following wavelengths were used D1 λ = 245.32 nm, and D2 λ = 243.30, whereas OXP was determined at D1 λ = 266.03 and D2 λ = 301.09 nm (Fig. 3).

Quantitative determination of LAB and SOT in a binary mixture was possible only by using the third derivative of the absorption spectrum (D3), and the selected wavelengths were λ = 249.91 nm for SOT and λ = 246.03 nm for LAB (Fig. 4).

Quantitative determinations of the mixture composed of OXP and SOT were carried out using the D1, D2 and D3 derivatives at wavelengths: for SOT D1 λ = 230.56 nm, D2 λ = 232.56 nm and D3 λ = 238.84 nm, while for OXP D1 λ = 245.32 nm, D2 λ = 240.18 and D3 λ = 232.05 nm, respectively (Fig. 5).

Method validation

The validation was done according to ICH recommendations for linearity, range, accuracy and precision, limit of detection (LOD), limit of quantification (LOQ) and relative recovery (20).

Table 2. Validation parameters of the proposed spectrophotometric methods for the determination of labetalol.

Parameters	Labetalol hydrochloride		
	D1 λ = 245.32 nm)	D2 (λ = 243.30 nm)	D3 (λ = 246.03 nm)
Linearity range [g/mL]	12.80 - 44.80		
Regression equation	y = 0.0014x + 0.0018	$y = 0.0001x - 0.2 \cdot 10^{-5}$	$y = 0.00002x + 0.4 \cdot 10^{-4}$
Correlation coefficient (r)	0.9979	0.9999	0.9977
Durbin-Watson test	1.278	1.647	1.317
Lagrange'a test (p)	3.188 (0.074)	-	3.474 (0.062)
Shapiro-Wilk test (p)	0.973 (0.910)	0.837 (0.122)	0.967 (0.871)
Mandel's test (p)	1.797 (0.272)	1.328 (0.333)	1.995 (0.253)
LOD [g/mL]	2.76	1.00	3.43
LOQ [g/mL]	8.37	3.11	10.39
Accuracy[a] [%]	100.25 ± 1.70	98.52 ± 1.14	100.87 ± 1.98
Precision[b] [%]	99.75	99.57	101.35
RSD%	1.69	1.16	1.97

[a)] Three concentrations of each analyte (20.48, 25.60, 30.72 µg/mL), repeated three times for each concentrate. [b)] Three concentrations of each analyte (12.80, 25.60, 38.40 µg/mL), repeated three times for each concentrate.

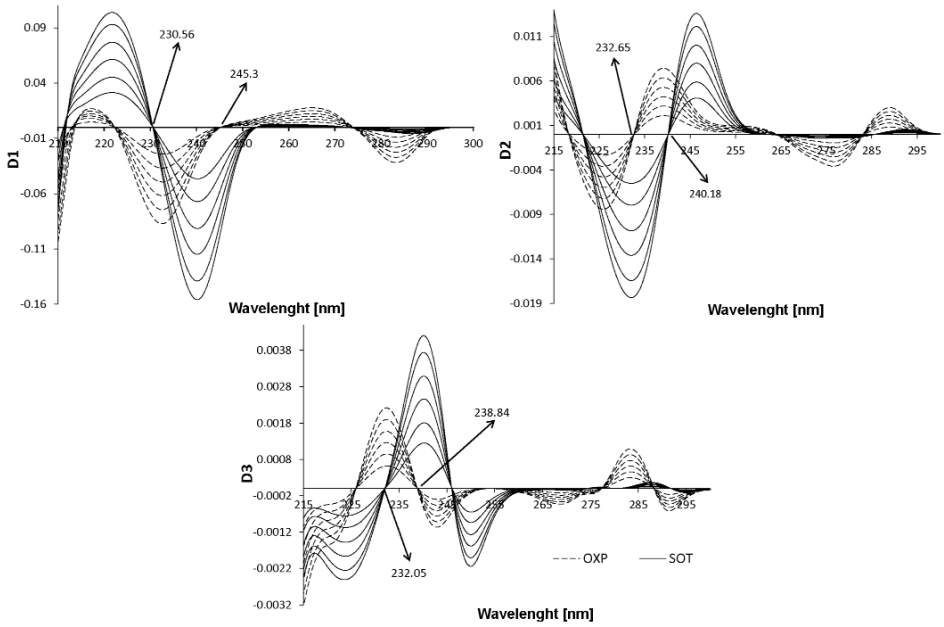


Figure 5. The first (D1), second (D2) and third (D3) derivative values for standard solutions of SOT (-) and OXP (——).

Table 3.Validation parameters of the proposed spectrophotometric methods for the determination of oxprenolol.

Parameters	Oxprenolol hydrochloride					
	D1(λ = 266.03 nm)	D1(λ = 245.32nm)	D2(λ = 301.09 nm)	D2(λ = 240.18 nm)	D3(λ = 232.05 nm)	
Linearity range [g/mL]	11.00 - 38.50					
Regression equation	y = 0.0004x - 0.0004	y = 0.0021x + 0.0016	y = 0. 00004x + 0.7·10^{-4}	y = 0.0002x - 0.3·10^{-4} y = 5.5·10^{-8}x^2 + 1.6·10^{-4}x - 1.1·10^{-5}	y = 0.00005x - 0.2·10^{-4}	
Correlation coefficient (r)	0.9999	0.9989	0.9982	0.9999	0.9999	
Durbin-Watson test	1.737	1.897	0.994	1.305*	1.790	
Lagrange'a test (p)	-	-	4.107 (0.043)	1.256 (0.262)*	-	
Shapiro-Wilk test (p)	0.977 (0.937)	0.980 (0.951)	0.973 (0.911)	0.903 (0.390)*	0.924 (0.536)	
Mandel's test (p)	0.527 (0.647)	1.083 (0.374)	1.121 (0.367)	0.349 (0.592)	0.024 (0.888)	
LOD [g/mL]	0.67	2.06	0.26	0.16	0.33	
LOQ [g/mL]	2.04	6.23	0.78	0.47	0.99	
Accuracy[a] [%]	100.73 ± 1.76	100.13 ± 1.86	101.32 ± 1.20	100.82 ± 1.29	100.22 ± 1.89	
Precision[b] [%]	101.23	99.85	102.45	100.54	99.75	
RSD%	1.75	1.86	1.19	1.28	1.88	

[a)] Three concentrations of each analyte (20.16, 25.20, 30.24 µg/mL), repeated three times for each concentrate. [b)] Three concentrations of each analyte (12.60, 25.20, 37.80 µg/mL), repeated three times for each concentrate. * for quadratic equation.

Linearity

Zero-order absorption spectra were registered for methanol solutions of SOT in the range of 11.0-38.5 µg/mL, for OXP in the range of 12.6-44.1 µg/mL and for LAB from 12.8 to 44.8 µg/mL. After conversion of the absorption spectra into the D1, D2 and D3 curves of derivative, the value of derivative was read at an appointed wavelength. The graph of D1; 2; 3 = f(c) was plotted. In the tested concentration ranges, linearity was maintained for all chosen wavelengths. To evaluate the results, linear regression equation characterizing the intersection points, correlation coefficients, Mandel's, Shapiro-Wilk, the Durbin-Watson and Lagrange tests were used.

In the evaluation of the calibration method, linear and quadratic fit were tested. Both models were compared using Mandel's test. The p-value < 0.05 indicates an equivalence of both fits. Normal distribution of the residuals was tested by the Shapiro-Wilk test. The value of W greater than the critical value allows to assume normal distribution. By using Durbin-Watson test, the presence of residual autocorrelation was checked. If the tested values of DW are greater than the upper critical value, no significant autocorrelation is observed, and if the DW values are lower than the lower critical value, then autocorrelation appears. The test does not give an answer on the occurrence of autocorrelation, when DW is in the range between the lower and upper critical value and in such case, the application of Lagrange test is recommended.

Accuracy and precision

The accuracy of the method was determined as a percentage of analyte recovery for the prepared solutions at three concentration levels: 80, 100 and 120%.

The precision of the method was checked using the proposed procedure for the determination of solutions containing very specific analyte amounts at three concentration levels: 50, 100 and 150%.

All measurements were repeated three times, accuracy and precision of the results were calculated using the appropriate regression equation.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were determined using standard error of the estimate (S_Y) and the slope of the calibration curve (a) and calculated according to the formulas:

$LOD = 3.3 \cdot S_Y/a$ and $LOQ = 10.0 \cdot S_Y/a$.

Laboratory prepared mixtures containing different ratios of SOT, LAB and OXP (Selectivity)

To a series of 5.0 mL volumetric flasks, different amounts of working solutions were accurately transferred and filled with methanol to a specified volume, obtaining the following mixtures: SOT-LAB, SOT-OXP and LAB-OXP at concentrations of 17.60, 22.00 and 26.40 μg/mL for SOT, 20.48, 25.60 and 30.72 μg/mL for LAB and 20.16, 25.20 and 30.24 μg/mL for OXP, respectively. For such binary mixtures of the active compounds, absorption spectra in the range 200-400 nm were recorded. After converting spectra into derivatives of an appropriate order, the values of derivatives against experimentally determined wavelengths were read. The amount of determined compound was calculated using an appropriate regression equation. Results along with the statistical evaluation are shown in Tables 1-3.

Table 4. Determination of the two analytes in laboratory prepared mixtures by the proposed spectrophotometric methods.

Conc [μg/mL]		SOT			OXP		
		(Recovery% ± S_x)					
		D1	D2	D3	D1	D2	D3
SOT	**OXP**						
24.20	16.38	102.43 ± 2.03 $t_{0.95}$ = ± 3.23 RSD = 1.98%	101.10 ± 0.77 $t_{0.95}$ = ± 1.22 RSD = 0.76%	102.52 ± 1.95 $t_{0.95}$ = ± 3.10 RSD = 1.90%	102.25 ± 0.77 $t_{0.95}$ = ± 2.97 RSD = 1.89%	100.62 ± 1.72 $t_{0.95}$ = ± 2.73 RSD = 1.70%	102.08 ± 4.86 $t_{0.95}$ = ± 7.73 RSD = 4.76%
19.80	22.68	102.15 ± 2.59 $t_{0.95}$ = ± 4.13 RSD = 2.53%	102.53 ± 2.83 $t_{0.95}$ = ± 4.50 RSD = 2.75%	101.83 ± 2.97 $t_{0.95}$ = ± 4.73 RSD = 2.91%	103.47 ± 1.52 $t_{0.95}$ = ± 2.42 RSD = 1.47%	104.85 ± 1.18 $t_{0.95}$ = ± 1.88 RSD = 1.12%	106.50 ± 1.00 $t_{0.95}$ = ± 1.55 RSD = 0.94%
17.60	32.76	100,88 ± 0,53 $t_{0.95}$ = ± 0.85 RSD = 0.52%	100.98 ± 2.85 $t_{0.95}$ = ± 4.53 RSD = 2.18%	103.06 ± 0.65 $t_{0.95}$ = ± 1.05 RSD = 0.63%	103.45 ± 1.36 $t_{0.95}$ = ± 2.16 RSD = 1.31%	102.07 ± 1.31 $t_{0.95}$ = ± 2.08 RSD = 1.28%	102.83 ± 1.07 $t_{0.95}$ = ± 1.70 RSD = 1.04%
		SOT			**LAB**		
SOT	**LAB**						
14.30	28.16			101.30 ± 1.78 $t_{0.95}$ = ± 2.83 RSD = 1.75%			100.43 ± 2.66 $t_{0.95}$ = ± 4.24 RSD = 2.65%
19.80	23.04			102.83 ± 1.35 $t_{0.95}$ = ± 2.11 RSD = 1.31%			98.32 ± 0.68 $t_{0.95}$ = ± 0.90 RSD = 0.69%
28.60	20.48			104.28 ± 1.24 $t_{0.95}$ = ± 1.97 RSD = 1.19%			95.52 ± 1.63 $t_{0.95}$ = ± 2.60 RSD = 1.70%
		LAB			**OXP**		
LAB	**OXP**						
16.64	27.72	99.66 ± 2.76 $t_{0.95}$ = ± 4.36 RSD = 2.76%	99.60 ± 4.27 $t_{0.95}$ = ± 6.71 RSD = 4.28%		99.76 ± 1.99 $t_{0.95}$ = ± 3.18 RSD = 2.00%	99.63 ± 1.88 $t_{0.95}$ = ± 3.00 RSD = 1.88%	
23.04	22.68	102.25 ± 1.84 $t_{0.95}$ = ± 2.94 RSD = 1.80%	100.88 ± 2.18 $t_{0.95}$ = ± 3.48 RSD = 2.16%		105.00 ± 1.02 $t_{0.95}$ = ± 1.40 RSD = 0.97%	103.20 ± 3.08 $t_{0.95}$ = ± 4.90 RSD = 2.98%	
33.28	20.16	102.95 ± 1.00 $t_{0.95}$ = ± 1.55 RSD = 0.97%	100.87 ± 0.92 $t_{0.95}$ = ± 1.45 RSD = 0.91%		101.88 ± 2.54 $t_{0.95}$ = ± 4.04 RSD = 2.45%	101.15 ± 1.77 $t_{0.95}$ = ± 2.78 RSD = 1.75%	

Table 5. Determination of the two analytes in spiked human serum by the proposed spectrophotometric methods.

Conc. [μg/mL]		SOT			OXP		
		(Recovery% ± S_x, n = 5)					
		D1	**D2**	**D3**	**D1**	**D2**	**D3**
SOT	**OXP**						
15.86	18.51	97.06 ± 1.06 $t_{0.95}$ = ± 1.35 RSD = 1.09%	98.06 ± 0.63 $t_{0.95}$ = ± 0.64 RSD = 0.78%	104.26 ± 0.21 $t_{0.95}$ = ± 0.25 RSD = 0.19%	101.74 ± 3.63 $t_{0.95}$ = ± 5.28 RSD = 4.13%	102.88 ± 4.26 $t_{0.95}$ = ± 0.75 RSD = 0.64%	100.12 ± 2.68 $t_{0.95}$ = ± 2.35 RSD = 2.67%
		OXP			**LAB**		
		D1	**D2**		**D1**		**D2**
OXS	**LAB**						
15.55	13.59	97.30 ± 2.75 $t_{0.95}$ = ± 3.35 RSD = 2.82%	98.10 ± 0.10 $t_{0.95}$ = ± 0.12 RSD = 0.10%		94.54 ± 2.45 $t_{0.95}$ = ± 2.55 RSD = 2.58%		99.22 ± 4.38 $t_{0.95}$ = ± 5.35 RSD = 4.41%
		SOT			**LAB**		
		D3			**D3**		
SOT	**LAB**						
13.21	9.58	101.34 ± 1.18 $t_{0.95}$ = ± 2.20 RSD = 1.78%			102.18 ± 3.60 $t_{0.95}$ = ± 3.35 RSD = 3.09%		

S_x - standard deviation. $t_{0.95\%}$ - confidence interval. RSD - relative standard deviation.

Preparation of real samples

Laboratory prepared mixtures

To a series of 5.0 mL volumetric flasks, appropriate amounts of standard solutions of examined substances were measured to obtain concentrations of components in the mixture corresponding to the ratio of 1 : 2, 1 : 1 and 2 : 1 and within the linearity range of calibration curve for the appropriate substance.

Human serum

To 2.0 mL Eppendorf tubes, 400 μL of human serum was added and the appropriate volume (50-125 μL) of standard solutions of examined drugs was measured to form binary mixtures at a given concentration of the determined substance that were vortexed for 1 min. To the prepared samples methanol was added so that the total volume of the mixture was 1.5 mL, and the mixture was vortexed for 3 min. After that, the mixture was centrifuged (10000 rpm for 5 min., temp. 22^{o}C). From each tube 500 μL of the supernatant was transferred to a series of 5.0 mL flasks and filled with methanol to the specified volume. For the samples prepared in this way, absorption spectra in the range 200-400 nm were recorded using blank prepared in the same way as sample solutions but without standard solutions. After converting obtained spectra into derivatives of an appropriate order, the values of derivatives against experimentally determined wavelengths were read. The concentration of the determined compound in the examined mixture was calculated using an appropriate regression equation. Results along with the statistical evaluation are shown in Tables 4, 5.

DISCUSSION

Hypertension is a growing medical problem worldwide. With an increasing number of people suffering from hypertension every year, the use of antihypertensive drugs such as β-blockers increases. The paper presents a procedure for the simultaneous determination of drugs from this class by derivative spectrophotometry. A characteristic feature of this method is its simplicity, speed, and the possibility of elimination of the interactions between determined substances and their influence on the determination results. In general, it was found that the proposed procedure enables the determination of substances from the group of β-blockers with satisfactory accuracy and precision both in pharmaceutical preparations and in the presence of human serum. The fact that the absorption spectra of the tested substances interfere with each other, causes that direct spectrophotometric analysis is impossible. It seems that the above-mentioned difficulties are the reason for replacing the spectrophotometric method by separation methods, which is justified in many cases.

Taking into account the advantages of the spectrophotometric method such as simple way of performing measurements, accuracy and availability as well as progress in the production of modern apparatus, we were able to demonstrate that this method has still a wide range of applications in fast and accurate quantitative analysis. New computational techniques create the potential for wider application of spectrophotometry, the application of derivatives of the zero-order spectra increases selectivity and sensitivity of analyses in comparison with the classical zero-order spectrophotometry.

Conducted measurements indicate, that the application of derivative spectrophotometry and conversion of spectra into first, second and third order derivatives, for the determination of sotalol, labetalol and oxprenolol, enable the simultaneous analysis of active compounds in binary mixtures and in human spiked serum.

Considering selected wavelengths, determined by the 'zero-crossing' method, it was demonstrated that the method is specific for the analyte, that is, tested compounds.

Quantitative determination carried out in mixtures and in the spiked serum was possible with the application of a corresponding order of the derivative, and so in case of the mixture OXP and LAB optimal results were obtained using D1 and D2 derivatives. For the quantitative determination of the mixture composed of OXP and SOT, D1-D3 derivatives may be used. If SOT and LAB were present in the sample, curves of derivatives limited the possibility of using only D3 derivative. No interference of matrix components was observed, which demonstrates a good selectivity of the method. The linearity was maintained over a wide range of concentrations between 11.00 and 38.50 μg/mL for sotalol, between 12.80 and 44.80 μg/mL for labetalol, and between 12.60 and 44.10 μg/mL for oxprenolol. The value of correlation coefficient R in the range of 0.9977–0.9999 did not specify clearly the linearity of calibration method. Therefore, to evaluate the linearity, Mandel's test was used. The obtained results of linear and quadratic fit, indicated a linear fit of the calibration curves in most cases, except for oxprenolol D2 240.18 nm and sotalol D1 320.56 nm where quadratic fit was chosen. The normality of distribution of residuals was confirmed by the Shapiro-Wilk test. The points of line intersection of the appropriate curves did not signifi-

cantly differ from zero. The results of the analysis of the Durbin-Watson test showed no significant autocorrelation of random components of calibration curves for OXP D1 245.43 nm, D1 266.03 nm, D3 232.05 nm, LAB D2 243.30 nm, SOT D1 230.56 nm, D2 232.65, D3 238.84 and D3 249.91 nm. In the case of OXP D2 240.18 nm, D2 301.09 nm, LAB D1 245.32 nm and D3 246.03 nm, the Lagrange test was used, as the Durbin-Watson test was not conclusive. In the linear alignment for OXP D2 301.09 nm, negative autocorrelation was found, however it was decided to apply the least squares method, accepting less efficiency of estimators.

The sensitivity of the developed method was high, LOD values for particular substances were estimated at 0.21-1.88 μg/mL for sotalol, 1.00-3.43 μg/mL for labetalol and 0.16-2.06 μg/mL for oxprenolol, whereas LOQ values were estimated at 0.65-5.70 μg/mL for sotalol, 3.11-10.39 μg/mL for labetalol and 0.47-6.23 μg/mL for oxprenolol depending on the composition of the mixture and on the class of derivative. Percent of the recovery of tested compounds presented as the average values for the three concentration levels was high and was in the range of 100 ± 5%. All validation parameters are summarized in Tables 1-3. The results of the measurements for particular active compounds both in mixtures and in spiked human serum did not differ from the declared values, they were characterized by good precision and accuracy, as evidenced by S_X - standard deviation, $t_{0.95\%}$ - confidence interval and RSD - relative standard deviation presented in Tables 4 and 5.

CONCLUSION

Proposed derivative spectrophotometric method is useful for the direct determination of sotalol, labetalol and oxprenolol both in binary mixtures and in spiked human serum. The application of „zero-crossing" technique allowed for the determination of the appropriate wavelengths, at which no interference of determined analytes was observed. The proposed method is precise, accurate and specific under experimental conditions. It can be used for routine determinations of tested substances as an alternative to time consuming and expensive separation techniques.

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REFERENCES

1. Zając M., Pawełczyk E.: Chemistry of drugs (in Polish), Akademia Medyczna im. Karola Marcinkowskiego, Poznań 2000.
2. Mutschler E., Geisslinger G., Kroemer K.H., Ruth P., Schaefer-Korting M.: Pharmacology and toxicology, ISBN: 978-83-7609-719-0, Polish Edition III, W. Buczko Ed., MedPharm Polska, Wrocław 2012.
3. Ogrodowczyk M.B., Marciniec B.: Acta Pol. Pharm. Drug Res. 70, 779 (2013).
4. Saleem K., Ali I., Kulsum U.: J. Chromatogr. Sci. 51, 807 (2013).
5. Feng Z., Yu S., Liu W., Yang L., Liu Y. et al.: Biomed. Chromatogr. 29, 1167 (2015).
6. Gu H.W., Wu H.L., Yin X.L., Li X., Liu Y.J. et al.: Anal. Chim. Acta 27, 10 (2014).
7. Caban M., Stepnowski P., Kwiatkowski M., Migowska N., Kumirska J.: J. Chromatogr. A 1218, 8110 (2011).
8. Xu L., Guo Q., Yu H., Huang J., You T.: Talanta 15, 462 (2012).
9. Yang J., Wang L., Guo Q., Yang G.: Se Pu 30, 280 (2012).
10. Al-Othman Z.A., Al-Warthan A., Alam S.D., Ali I.: Biomed. Chromatogr. 28, 1514 (2014).
11. Huang L., Lin J.M., Yu L., Xu L., Chen G.: Electrophoresis 29, 3588 (2008).
12. Aturki Z., D'Orazio G., Rocco A., Fanali S.: Electrophoresis 32, 2602 (2011).
13. Ma Y., Zhang H., Chen H., Chen X.: Electrophoresis 35, 3345 (2014) .
14. Santos M.G., Tavares I.M., Boralli V.B., Fiqueiredo E.C.: Analyst 140, 2696 (2015).
15. Musenga A., Cowan D.A.: J. Chromatogr. A 1288, 82 (2013).
16. Abdel Hameed E.A., Abdel Salam R.A., Hadad G.M.: Spectrochim. Acta A 141, 278 (2015).
17. Stolarczyk M., Maślanka A., Krzek J., Milczarek J.: Acta Pol. Pharm. Drug Res. 65, 275 (2008).
18. Stolarczyk M., Maślanka A. , Apola A., Krzek J.: Acta Pol. Pharm. Drug Res. 70, 967 (2013).
19. Stolarczyk M., Maślanka A., Apola A., Rybak W., Krzek J.: Spectrochim. Acta A 148, 66 (2015).
20. ICH-Q2 (R1) Validation and Analytical Procedures: Text and Methodology. International Conference on Harmonization: Geneva, November 2005.

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